A METHOD OF TRANSMITTING BLOOD PARASITES*

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In chemotherapeutic researches involving the experimental infection of large numbers of white rats with T. equiperdum, T. brucei, T. lewisi, and Sp. recurrentis, it was found that the usual methods of obtaining a small amount of blood from an infected or seed rat by snipping the tail and bleeding into sodium citrate solution or defibrinating the blood with glass beads in a test tube were unsatisfactory because of the danger of contaminating the blood with various microorganisms during the operation.

I have found the following method very serviceable. By this technic sufficient blood is obtained from the heart in an aseptic manner to infect a large series of rats without killing or seriously injuring the stock or seed animal. For the purpose of propagating a strain of trypanosomes by infecting one or two rats at regular intervals, the method has been found likewise serviceable, as a small amount of blood may be obtained aseptically and at frequent intervals without injury to the stock animal.

The seed rat is fastened to an operating board or held by a gloved assistant and the cardiac area determined by palpation, as when bleeding a guinea-pig from the heart. Owing to the rapid beating of the heart and thinness of the thoracic wall, this is easily and quickly determined.

One or two applications of tincture of iodin (10 percent) are made over the cardiac area to sterilize the hair and skin.

A test tube of sterile 1 percent sodium citrate in normal salt solution is warmed by heating in a Bunsen flame and a sufficient amount drawn carefully into a sterile 1 or 2 c.c. Record syringe, fitted with a medium-sized needle (No. 22), in such a manner as to avoid contamination. The 1 c.c. syringe is employed when few injections are to be made; for the purpose of infecting a larger series of rats, as thirty or more, a 2 c.c. syringe is used. The syringe is not entirely filled with the citrate-salt solution, but sufficient space is permitted

* Received for publication January 22, 1915.

for suction by withdrawal of the piston when the needle has been passed into the heart.

The skin over the cardiac area is drawn taut by the forefinger and thumb of the left hand without touching the site of puncture and the needle quickly entered into the heart, directed slightly upwards and toward the spine. No attempt is made to enter any particular chamber of the heart.

At once, blood flows into the syringe. The action may be facilitated by gentle suction, altho usually this is not necessary. When sufficient blood is obtained the needle is quickly withdrawn, the contents of the syringe are mixed by gentle agitation, and the animal returned to the cage. With heavily infected rats showing large numbers of trypanosomes or spirilli in every microscopic field, I secure a dilution of blood equivalent in density to a 1 percent suspension of erythrocytes. If the number of parasites in the blood of the seed rat is fewer in number, a correspondingly heavier emulsion is prepared. A heavy emulsion may be diluted by drawing into the syringe a sufficient amount of sterile normal salt solution (warmed) or by expelling the contents of the syringe into a sterile test tube and diluting as desired. Fresh rats are then infected by the intraperitoneal injection of 0.1-0.2 c.c. of the emulsion.

By this method the whole procedure is quickly conducted in a sterile manner and only in exceptional instances is the animal seriously injured or killed by the operation. It is important to use a sharp-pointed needle, and one not so large as to unnecessarily injure the heart and not so small as to hinder the flow of blood.